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Studies on Mineral and Protein

Utilization by Atlantic Salmon (*Salmo salar*) grown in Sea Water

S.P. Lall and F.J. Bishop

Disease and Nutrition Section
Fisheries and Environmental Sciences Division
Resource Branch,
Department of Fisheries and Environment
Halifax, Nova Scotia B3J 2R3

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ABSTRACT

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The present report summarizes the results of four experiments designed to study mineral and protein utilization by Atlantic salmon. (Salmo salar) grown in seawater. These nutrients are discussed separately in two sections. The first section deals with the utilization of calcium, phosphorous, magnesium, sodium, potassium, cobalt, copper, manganese, zinc, iron, iodine and fluorine from seawater and feed. The response was measured in terms of growth, feed utilization, bone ash content, hematocrit, hemoglobin and mineral level in blood. Results indicate that fish can derive calcium from seawater provided sufficient phosphorous is present in the diet. The addition of sodium, potassium and magnesium was also not necessary in the diet. However, phosphorous and trace element (zinc, copper, cobalt, iron, manganese, iodine and fluorine) supplementation was required for optimum growth, food utilization, bone mineralization and normal hemoglobin, hematocrit and mineral levels in the blood.

The second section consisted of experiments on protein requirements, effects of varying calorie and protein ratios in the diet and the digestibility of various proteins in seawater. Based upon growth, feed efficiency, mortality, body composition and liver composition data, the protein requirement of Atlantic salmon was estimated to be approximately 45 g protein per 100 g of dry diet. Genrally, the increase in dietary fat and protein level improved the growth and feed efficiency. Diet containing low protein, high carbohydrate and high fat resulted in increased liver glycogen deposition. A dietary balance of carbohydrate, lipid and protein was essential to overcome the high liver glycogen accumulation. Growth and biochemical data suggest that diet containing 45% protein and 20% fat showed the optimum response.

The digestibility of casein, fish protein concentrate (FPC), soybean protein and fish meal was estimated employing an indirect method using chromic oxide as digestion indicator. Casein (94.1%) was digested significantly better than FPC (91.5%), soybean protein (86.4%) and fish meal (77.7%). Increasing the level of these proteins in the diet resulted in a decrease in protein digestibility. The reduction in protein digestibility was more pronounced with fish meal and soybean than FPC and casein.

RÉSUMÉ

Lall, S.P. and F.J. Bishop. 1976. Studies on mineral and protain utilization by Atlantic Salmon (Salmo salar) grown in seawater. Fish. Mar. Serv. Res. Dev. Tech. Rep. 688: 16 p.

Le présent rapport résume les resultats de quatre expériences concues pour étudier l'utilisation des sels mineraux et des protéines chez le saumon de l'Atlantique (Salmo salar) élevé en eau de mer. Ces éléments nutritifs sont traités séparément dans deux sections. La première section traite l'utilisation du calcium, phosphore, magnésium, sodium, potassium, cobalt, cuivre, manganese, zinc, fer, iode et fluor provenant de l'eau de mer et de l'alimentation. Les effets ont été mésurés en suivant la croissance, l'utilisation alimentaire, la quantité de cendre dans les os, le volume moyen de cellules rouge concentrées, et les concentrations de l'hémoglobine et des minéraux dans le sang. Les résultats indiquent que ces poissons peuvent tirer du calcium de l'eau de mer pourvu qu'une quantité suffisante de phosphore soit présents dans le régime. L'addition à la nourriture du sodium, du potassium et du magnésium n'était pas nécessaire. Cependant, l'addition à la diète du phosphore et des traces d'éléments (zinc, cuivre, cobalt, fer, manganese, iode et fluor) était nécessaire pour obtenir l'optimum de la croissance, de l'utilisation alimentaire et de la minéralisation des os, ainsi que pour obtenir des niveaux normaux de l'hémoglobine, du volume moyen de cellules rouges concentrées et des minéraux dans le sang.

La deuxième section décrit des expériences sur les besoins en protéines, sur les effets de divers rapports calories/protéines dans le régime, et sur la digestibilité de diverses protéines dans l'eau de mer. D'après les données sur la croissance, l'éfficacite alimentaire, la mortalité et la composition du corps et du foie, les besoins en protéines du saumon de l'Atlantique ont été estimés approximativement a 45 g de protéines par 100 g de nourriture sèche. En général, la croissance et d'éfficacité alimentaire se sont améliorées avec l'augmentation des niveaux de matierès grasses et de protéines. De L'alimentation composée de bas niveaux de protéines et de hauts niveaux d'hydrates de carbone et de matières grasses a produit une de'pôt augmentée du glycogène dans le foie. Un régime équilibre en hydrates de carbone, lipides et protéines était nécessaire pour surmonter cette grande accumulation de glycogène dans le foie. Les données sur la croissance et sur la biochimie suggèrent qu'un régime contenant 45% de protéines et 20% matières grasses a produit la réaction optimale.

La digestibilité de caséine, de concentré de protéine des poissons, de protéine de fève soya et de la farine de poisson a été estimee avec une méthode indirecte qui emploie l'oxyde de chrome comme indicateur de la digestion. La digestion de caséine (94.1%) était significativement meilleure que celle du concentré de protéine des poissons (91.5%), de protéine de fève soya (86.4%) et de la farine de poisson (77.7%). L'augmentation de concentration de ces protéines dans le régime a produit une diminution de la digestibilite des protéines. Cette réduction de la digestibilite était plus prononcée avec la farine de poisson et la protéine de fève soya qu'avec le concentré de protéine des poissons et de la caséine.

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I. GENERAL INTRODUCTION

In recent years considerable interest has developed for marine aquaculture of salmonids in the Maritimes, because of the limited supply from natural stock. Before large production of fish can become an economic reality, a substantial amount of research is needed in the areas of selective breeding, disease control, management and nutrition of salmonids. A full understanding of the nutritional requirements and the factors influencing food conversion is important because feed represents the largest single cost item in production. Unfortunately, little definitive work has been done on the nutrient requirements of fish in seawater.

Salmonids are noted for their efficient conversion to protein. If fish feed became better formulated, the yields of protein from marine fish farming could be as high as those from plant protein. In order to develop successful feed formulas, basic information is required on the nutrient requirements, the organoleptic properties of feed in relation to acceptability and ability of fish to digest and utilize nutrients from various sources. The physical form in which food is fed has large effects on feed intake and utilization. A correct consistency and particle size would reduce the amount of food required for conversion into flesh. Moreover, the addition of pigment to the feed could produce attractively colored flesh.

The nutritional requirements of salmonids should be considered at various stages of development i.e. parr, smolt and post-smolt stage (seawater). Most studies on nutritional requirements and food formulation are confined to freshwater salmonids (up to parr stage). Recently The National Academy of Sciences published a bulletin listing the nutritional requirements for salmon, trout and catfish.

Atlantic salmon (Salmo salar) go through a characteristic physiological change during the smolt stage. It has been demonstrated that photoperiod, water temperature and diet affect smoltification. Studies on the relationship between nutrition and smoltification or the rate of survival during the salt water adaptation stage are limited. Zaugg (1969) reported that salt water supplementation of chinook salmon (Oncorhynchus kisutch) diet resulted in an increase in gill ATP-ase activity and consequently better survival of fish in seawater. Some of the work conducted by the authors indicates that type of lipid, protein level and mineral content of diet and the water temperature have a significant effect on the survival of Rainbow trout and Atlantic salmon in seawater. Normally, it takes about two years to produce Atlantic salmon smolts in Maritime hatcheries. The possibility of reducing the time period and inducing early smoltification by dietary manipulation is a formidable challenge.

In a marine environment, fish can derive a number of minerals from the saline environment to satisfy specific needs for growth and metabolism and may not require their addition in the diet. However, the information on minerals as well as protein, amino acids, lipid, energy and vitamin requirements of salmonid in seawater is limited.

The present report is the summary of four experiments designed to investigate mineral and protein utilization by Atlantic salmon in seawater. Each nutrient has been discussed separately in two sections. The first section deals with the utilization of macro and micro elements from seawater and feed. On the basis of results from this study, minerals can be divided in two categories (1) those which can be derived from seawater and (2) others which require dietary supplementation. The findings of this study will be helpful in developing test diets to determine the requirements of individual minerals as well as in diet formulations.

The second section consists of experiments on protein requirements, response of varying calorie to protein ratios in the diet and the digestibility of various proteins in seawater. The results reported here will be useful in practical diet development for marine aquaculture. Furthermore some basic information has been gained on carbohydrate, lipid and protein utilization in relation to food intake.

II. MINERAL UTILIZATION IN SEAWATER BY ATLANTIC SALMON

A. INTRODUCTION

Studies on mineral requirements of salmonids grown in seawater and freshwater are limited. Most of the research on inorganic elements is confined to osmoregulation and toxicity. Recently, some workers (Andrews et al., 1973. Katola, 1975) have demonstrated the calcium and phosphorous requirements of catfish (Ictalurus punctatus) and Atlantic salmon (Salmo salar) in freshwater. Evidence also indicates that dietary minerals have significant effects on growth, survival and bone development of Oncorhynchus tshawytscha (Woodall and LaRoche, 1964), Chrysophrys major (Sakamoto and Yone, 1973), Seriola quinqueradiata (Ikeda et al., 1970), Cyprinus carpio and Salmo gairdneri (Ogino and Kamizono, 1975).

In a marine environment, salmonids maintain water and salt balance by ingesting seawater and excreting sodium, potassium and chloride through gills. Thus the intake of minerals through seawater may reduce their addition in the diet. Yone et al., (1974) observed that the removal of calcium from the diet had no effect on growth of Chrysophrys major. Phillips (1959) indicated that the amount of calcium absorbed from freshwater varies with the quantity of calcium present in the diet. Low dietary calcium caused an increase in the amount absorbed from the water, and high dietary calcium reduced the uptake of calcium from water.

The present investigation was designed with the following objectives: 1. To determine if calcium supplementation is necessary in a diet containing an adequate level of phosphorous for Atlantic salmon grown in seawater; 2. To determine if it is essential to supplement the diet of Atlantic salmon with magnesium, manganese, zinc, iron, copper, cobalt, sodium, potassium, iodine and fluorine in marine environment; 3. To determine whether the combination of above minerals shows any growth response; 4. To determine the effects of these dietary inorganic elements on body composition, hematology and serum mineral levels of Atlantic salmon.

B. MATERIALS AND METHODS

The composition of the basal diet and the amount of mineral added to each diet are shown in Tables 1 and 2 respectively. All diets were

isonitrogenous and isocaloric. The following dietary mineral combinations were used.

DIET NO.	MINERAL SUPPLEMENT
1	None
2	Ca + P
3	Mn + Zn + Cu + Co + Fe + I + F (Trace elements)
4	P + trace elements
5	Ca + P + trace elements
6	Ca + P + Mg + trace elements

Calcium phosphate ($CaH_4(PO_4.H_2O)$) and calcium carbonate ($CaCO_3$), supplied the calcium and phosphorous in Diets 2, 5 and 6; whereas sodium phosphate ($NaH_2PO_4.H_2O$) and potassium phosphate (KH_2PO_4) were the main source of phosphorous in Diet 4. In addition to the mineral supplement 0.03% calcium and 0.3% phosphorous were derived from feed ingredients in each diet. The wet diets (50% water) were prepared by mixing the dry portion of the diet with hot (60°C) water. The gel that formed upon cooling was passed through a meat grinder to obtain the desired size of pellet. The diets were prepared every two weeks and stored at -20°C until fed.

Approximately 12 to 14 cm Atlantic salmon were distributed in 12 lots of 50 fish each. The weight of each lot was adjusted so that each lot contained an equal weight of fish. The fish were held in 0.91 m diameter aquaria. All fish were gradually acclimated to seawater for three weeks. The water temperature was maintained between 15-16°C throughout the experiment in all aquaria. The salinity of seawater was 32 ppt. Water temperature was monitored daily and the pH, dissolved oxygen and the total nitrogen measured periodically. A record of mortality was also maintained and the dead fish were examined for gross abnormalities.

After the saltwater adaptation and a two week adjustment period, duplicate lots of fish were fed each of the experimental diets for 16 weeks. Fish were fed all they would eat 3 times a day. They were group weighed every four weeks. At the end of the experiment, samples were taken for the determination of body composition and blood analyses.

Nitrogen in feed and carcass was determined on an automated Kjeldahl analyser (Technicon Instrument Co. Ltd.) according to the method of Ferrari (1960). The method of Bligh and Dyer (1959) was used for body lipid determination. Both carcass and bone ash were determined by A.O.A.C. (1970) procedure. Serum were analysed for calcium, magnesium, manganese, copper, cobalt, zinc and iron using Atomic Absorption Spectrophotometer (Perkin Elmer, Model 403). The hemoglobin content of fish blood was determined by the cyanomethemoglobin method (Wintrobe, 1956) and using the correction factor of Larsen and Sniessho (1961).

C. RESULTS

The data presented in Table 3 clearly indicate that Diet 4 containing 0.6% phosphorous and trace element supplement had the highest growth and feed efficiency. Fish fed with both calcium and phosphorous with or without trace element supplements (Diet 2, 5 and 6) showed lower weight gain as compared with those receiving Diet 4. Salmon fed only the trace element supplement (Diet 3) had the lowest growth, however, the feed efficiency was not significantly affected. The diet containing all the minerals (Diet 6) showed no improvement in growth over Diet 4. This would indicate that if sufficient phosphorous, zinc, iron, manganese, cobalt and copper, iodine and fluorine are present in the diet, supplementation of calcium, magnesium, sodium and potassium is not necessary for maximum growth and feed efficiency of Atlantic salmon in seawater.

Fish fed diet with no mineral supplement (Diet 1) showed relatively good growth. However, the body composition data (Table 4) revealed significant lower ash level as compared with remaining diets, indicating that bone mineralization was adversely affected. Addition of trace elements (Diet 3) alone showed an improvement in ash content over Diet 1. The maximum ash was noted in fish fed Diets 4, 5 and 6. However, the differences among these diets were not significant. Sufficiently good growth on Diet 1 containing no mineral supplement must be due to the utilization of minerals present in dietary ingredients. It is likely that the duration of the experiment was not long enough to cause a severe depletion of minerals from the body and consequently, complete reduction in growth rate.

The gross examination of selected fish from each dietary group failed to show any abnormality. It is also interesting to note that mortality (Table 3) was relatively low in all groups. Generally, fish died due to physical injuries. Some mortality during the early part of the experiment was attributed to the failure of salmon to adapt to seawater.

Serum mineral levels (Tables 5 and 6) were significantly affected by the dietary treatments. The lack of calcium and phosphorous supplements in Diets 1 and 3 reduced the serum calcium and phosphorous level with the magnitude of decrease being greater in Diet 1 than Diet 3. The highest serum phosphorous was observed in fish fed Diet 4. The difference in either serum calcium or phosphorous between Diets 2,4,5 and 6 was not significant. It appears that an adequate phosphorous intake was essential to attain the maximum serum calcium and/or phosphorous levels.

There was no consistent change in serum sodium, potassium and magnesium levels. Unlike other minerals, supplementation of diet with sodium, potassium and magnesium did not increase their serum content. Addition of trace elements supplement (Zn, Fe, Co, I, F, Cu and Mn) was essential to attain the optimum levels of these inorganic elements in the serum (Table 6). Absence of trace element supplements in Diet 1 and 2 was reflected in their lower serum levels. Trace elements together with calcium, phosphorous, magnesium, sodium and potassium did not significantly alter the level of serum trace elements.

Hematocrit and hemoglobin levels (Table 7) were also reduced by the deletion of trace elements from the diets (Diets 1 and 2). However, the serum protain level was unaffected. The importance of copper, cobalt and iron in red blood cell formation is well recognized. The deficiency of these minerals in the diet must have resulted a decrease in red blood cell formation and consequently lower hematocrit and hemoglobin concentration. It would appear that fish were unable to derive trace elements from seawater, and their supplementation in the diet was essential to maintain the normal health of fish.

D. DISCUSSION

It is well known that salmonids in freshwater do not drink water, however, in a marine environment they ingest seawater to replace the water loss resulting from dehydration. Seawater contains high concentrations of sodium, potassium, calcium and magnesium. Parry (1966) reported that most of the sodium, potassium and chloride present in seawater was readily absorbed in the intestine. Shehadeh and Gordon (1969) observed that during passage through the intestine, ingested seawater was gradually depleted of monovalent ions and calcium and enriched in magnesium and sulfate. Hickman (1968) also reported that the greater part of the calcium was absorbed in the gut and excreted through the gills because a very small amount of calcium was present in urine and rectal contents. It appears that fish fed diet containing no calcium supplement must be able to derive calcium from seawater in the above manner to meet the specific needs of growth and metabolism. Similarly, addition of sodium, potassium and magnesium to the diet does not show any growth response.

Results indicate that supplementation of phosphorous in the diet was essential for maximum growth response and calcium retention in the body. Podoliak (1961) found that brook trout (Salvenlinus fontinalis) efficiently absorbed phosphorous from the digestive tract in freshwater. Phillips et al. (1957) observed that brook trout have limited ability to absorb phosphorous from the water. Because trout held for 24 hours in water containing 1 mg of inorganic phosphorous per liter accumulated less than 0.4 µg of phosphorous directly from the water, it is apparent that phosphorous present in dietary ingredients was efficiently utilized; however, the additional supplement (Diet 4) was necessary for the bone mineralization and normal serum calcium and phosphorous levels. More work is required to establish the phosphorous requirement of Atlantic salmon in seawater.

It appears that food is the major source of Zn, Co, Cu, Mn, Fe and I for Atlantic salmon grown in seawater. Deletion of these minerals from the diet showed a significant reduction in their serum levels. Furthermore, hematocrit and hemoglobin levels were also reduced. An early stage of anemia was apparent. Normally, the concentration of the above mentioned minerals is relatively low in seawater (Macaulay, 1974). Pentreath (1973a) reported that plaice (Pleuronectes platessa) obtains zinc from diet rather than directly from water. Fletcher et al. (1975) also indicated that sockeye salmon (Oncornhynchus nerka) derived copper and zinc mainly from the natural food sources.

The present study has demonstrated the need of phosphorous, zinc, copper, cobalt, iron, manganese, iodine and fluorine in the diet of Atlantic salmon grown in seawater. Additional work is necessary to establish the requirements of individual minerals and their availability in feedstuffs. Any investigation on quantitative mineral requirements of fish encounters greater difficulty because of the exchange of salts across the gill membranes and skin. Consequently, the uptake and excretion of these elements cannot be measured precisely. Moreover, many metabolic and absorptive interrelationships among the mineral elements contribute to variation in the degree of physiological response to deficient and toxic levels. The genetic differences, age, sexual maturity and hormonal balance should also be considered in determining the mineral requirements.

III. PROTEIN UTILIZATION BY ATLANTIC SALMON IN SEAWATER.

A. INTRODUCTION

Most marine fish are carnivorous and require a high level of protein. The protein and amino acid requirements of salmonids in seawater is not well understood. Zeitoun et al. (1974) reported that the protein requirement of Rainbow trout increased with the increase in water salinity (10 to 20 ppt). Lall and Bishop (1976) found that approximately 50% protein was required in the diet of Rainbow trout for maximum growth, feed efficiency and survival in seawater. They also observed that the levels of taurine, β- alanine, aspartic acid, glutamic acid and glycine were significantly lower in saltwater fish. The essential amino acid requirement of salmonid in marine environment has yet to be established.

Several factors must be considered in determining the protein and amino acid requirement of the species in question. The influence of dietary protein level on utilization of protein has long been established, both in growth and in nitrogen balance studies with mammals. The utilization of protein decreases as its levels in the diet increases. Many factors other than the level of protein also affect the utilization of protein e.g. species, strain, age and sex of animals, type of protein, water temperature and salinity etc. Difference in the amino acids requirements among species and availability of amino acid in question can also affect the utilization of protein.

Energy is probably one of the most important factors to consider when investigating the nutritive value of protein but also the calorie to protein ratio of a diet has long been stressed with other species. The protein requirements of animals may be spared by non-protein feedstuffs because of their calorific value. The low energy diets results in an increased catabolism of labile protein to overcome the energy deficiency in metabolic pool. Whereas high energy diets may lead to increased protein retention.

Investigations on the protein and energy needs of Atlantic salmon have been completely lacking. It is generally accepted that fish rely to a large extent on lipids and protein rather than carbohydrate for energy. Whether protein calories are used for catabolic or anabolic purposes is dependent upon the quality of protein for fish. Phillips (1948) reported that carbohydrate and fat could partly replace the dietary protein but the higher level of carbohydrate resulted in high blood sugar levels and an accumulation of glycogen in the liver of fish. In contrast, Buhler and Halver (1961) found that Chinook salmon can utilize high levels of carbohydrate (48% of the diet) without apparent deleterious effects and concluded that dietary imbalances were partially responsible for the results of Phillips et al. (1948) However, the fish receiving the high carbohydrate diet showed glycogen deposition in the liver.

The present study was designed to investigate the protein utilization by Atlantic salmon in seawater. A series of three experiments, were conducted with the following objectives: 1. To determine the protein requirement of Atlantic salmon in seawater. 2. To determine the effect of varying calorie and protein ratios on growth, feed effeciency, body composition, and serum glucose and protein levels of Atlantic salmon grown in seawater. 3. To determine the differences in protein digestibility of fish meal, fish protein concentrate (FPC), soybean

protein and casein.

B. EXPERIMENT 1

The first experiment was designed to determine the effect of various levels (35, 40, 45, 50 and 55%) of protein on growth, feed efficiency, body composition of Atlantic salmon grown in seawater. Casein and gelatin were the major source of protein. Diets were supplemented with arginine, phenylaline, cystine and methionine to overcome the marginal deficiency of these amino acids.

General experimental procedure employed in this experiment was essentially the same as the previous experiment (page 1). This includes type of fish, size, salt water adaptation procedure, method of diet preparation, randomization procedure for fish, fish holding facility, frequency of feeding, chemical analyses, sampling procedure and statistical procedure, etc. Both the experiments were conducted simultaneously.

Duplicate lots of 30 fish were fed each of the five experimental diets (Table 7) for 12 weeks. They were group weighed every four weeks. At the termination of the experiment, samples were taken for the determination of body and liver composition. The liver nitrogen was determined on Autoanalyzer by microkjeldahl procedure. Liver glycogen was determined according to the method of Montgomery (1957).

The results of this experiment are summarized in Tables 9 to 11. The weight gain data indicate that growth was not greatly affected by the dietary protein level. The maximum growth was noted for fish fed 55% protein. The difference in weight gain of fish fed 35 to 50% protein was not significant. However, the feed efficiency showed a gradual improvement in feed conversion with the increase in the protein content of the diet. The relatively good growth of fish fed a diet containing 35% protein may well be explained on the basis of a good balance of amino acids in the diet.

Generally the mortality was low in all groups. The level of protein intake had no significant effect on the mortality. Gross examination of dead fish did not reveal any obvious changes in any of the organs e.g. liver, kidney, intestine and gill, etc. Most deaths were due to the body injuries resulting from aggressive feeding behaviour and the handling during monthly weighings.

The effects of various diets on body composition of salmon are shown in Table 10. Dietary protein had no significant effect on the moisture content. A slight increase in protein deposition was noted with the increase in protein content of the diet. Cowey et al. (1972) also observed that carcass protein content increased with the dietary protein level in plaice (Pleuronectes platessa).

Fish fed 40% protein showed higher lipid deposition as compared with those receiving diet containing 35% protein. A further increase in dietary protein content showed a gradual decrease in carcass lipid. Fish fed 35% protein showed lower ash than those receiving higher protein diets. But the differences in ash content between Diets 2, 3, 4 and 5 were not significant.

Effect of various experimental diets on liver composition of Atlantic salmon is shown in Table 11. Like body composition data, liver protein content increased with the dietary protein level. However, liver lipid data revealed that only the diet containing 40% protein showed an increase in liver lipid content with the change in dietary protein level. The difference in liver lipid between the remaining diets (No. 1, 3, 4 and 5) were not significant. Since the fat level remained constant in all the diets, changes in the dietary protein level had no effect on liver lipid deposition.

On the basis of growth, feed efficiency and carcass and lipid composition data, it seems reasonable to conclude that diet containing 45% protein produced the optimum response. Although fish fed diet containing 35% protein showed relatively good growth, bone mineralization was poor as compared with other experimental groups. Furthermore, fish fed diet containing 35 and 40% protein showed high liver glycogen and lipid deposition as compared with the remaining groups.

C. EXPERIMENT 2

The second experiment was designed to study the effect of varying the dietary energy level on protein utilization. A desired calorie and protein ratio in the diet insures that a substantial amount of protein is not utilized for energy. Dextrin and herring oil were the respective carbohydrates and lipid sources. Nine experimental diets (Table 12) containing three levels of protein (35, 45 and 55%) and three levels of lipid (8, 16 and 20%) were evaluated in terms of weight gain, feed efficiency, mortality, body composition, liver composition, serum protein and serum glucose level of Atlantic salmon.

The experiment consisted of a factorial arrangement in a randomized complete block design. Duplicate lots of 30 fish received various experimental diets for 12 weeks. General experimental procedure and analytical techniques were similar to those used in previous experiments. The data was analyzed by the method of analysis of variance. The studentized form of the extreme mean square test (Nair, 1948) was used for the significance of the treatment mean square appropriate to individual degrees of freedom. All tests of significance were made at the five percent level of probability.

The data presented in Table 13 suggest that the increase in the dietary protein level resulted in an improvement in weight gain. However, the magnitude of growth enhancement was related to the fat content of the diet. All except Diet 2 showed an increase in weight gain with the increase in dietary fat level. Generally, the feed conversion showed an improvement with the increase in protein and/or fat level.

The results (Table 14) indicate that dietary lipid had a significant effect on body composition. The increase in fat content of the diet resulted in higher body lipid and lower body moisture. This response was consistent with fish feed either 35, 45 or 55% protein. Low protein diets caused a decrease in body protein with the increase in dietary fat level. This effect was not evident with fish fed diet containing 45 and 55% protein. Salmon fed the high protein diets showed a decrease in body ash with the increase in dietary lipid. However, a similar decrease was not observed with the remaining groups.

Liver composition data (Table 15) indicate that the increase in dietary protein level resulted in higher protein deposition but the increase in dietary lipid showed no such response. Liver lipid showed a slight increase when the dietary lipid was raised to 14%. Diet containing 20% lipid showed higher liver lipid deposition only in groups fed 55% protein. Liver glycogen was greatly influenced by the carbohydrate content of the diet. Fish fed low protein diet showed a linear increase in liver glycogen content with the increase in dietary lipid. Salmon fed 45 and 55% protein showed no change in liver glycogen when dietary lipid was increased from 8 to 14%. However, the increase in dietary lipid from 14 to 20% showed a higher liver glycogen.

Effect of various treatments on serum protein level is summarized in Table 15. Generally the serum protein was slightly affected by the changes in the dietary protein and lipid levels. Low protein diet showed the highest serum protein level regardless of the fat content in the diet. At 14% dietary fat level, fish fed 55% protein diet showed higher serum protein than those given 45% protein but this response was reversed at 20% fat level. The serum protein increased with the increase in dietary fat from 8 to 14% reagrdless of the protein content of the diet.

Serum glucose level was significantly affected by the changes in dietary protein and fat contents. Increase in dietary protein resulted in a reduction in serum glucose level. This decrease in serum glucose was an indication of the dietary carbohydrate level because the protein was increased at the expense of carbohydrate. Thus low protein diets contained high levels carbohydrates and vice versa. Increase in dietary fat content resulted in higher serum glucose levels of fish fed 35 and 45% protein whereas 55% protein caused a decrease in serum glucose of fish fed high fat diets. It appears that dietary fat might have affected the carbohydrate absorption and consequently the serum glucose level.

It is apparent from the results that Atlantic salmon fed a nutritionally adequate diet can tolerate as high as 27.5% carbohydrate (dextrin) without any apparent deleterious effects. High carbohydrate diets (Diets 1 to 3) produced good growth without any apparent increase in mortality or gross pathological changes. However, diets (Diets 2 and 3) containing high fat and carbohydrate and low protein resulted in an increase in liver glycogen and blood glucose level. The dietary fat had no such adverse effects on liver glycogen and serum glucose when the dietary carbohydrate level was reduced. This would indicate that an optimum balance of carbohydrate, protein and fat was essential for maximum growth and efficient energy utilization.

The high level of dietary fat exerted a beneficial effect on growth and food conversion. It appears that Atlantic salmon can tolerate much higher levels of fat than other animal species without any apparent adverse effects. A balance of carbohydrate and fat in the diet was essential in the diet to overcome the increased liver glycogen deposition. High protein diets showed increased growth and feed efficiency but the level of fat played an important role in achieving the maximum response. It appears that if the proper nutrient balance is maintained, diet containing 45% protein and 20% fish oil would produce optimum growth and feed efficiency in Atlantic salmon.

D. EXPERIMENT 3

The present experiment was designed to compare the digestibility of casein, soybean meal (alpha protein), fish protein concentrate (FPC) and fish meal by Atlantic salmon. The above ingredients were incorporated into a semi-purified diet at two different levels to provide 35% and 45% protein. Casein, FPC, soybean meal and fish meal contained 92, 88, 80 and 71% protein respectively.

Approximately 25 to 28 cm Atlantic salmon (250 - 300 g) were divided into 16 groups each consisting of 25 fish. Fish were fed a standard test diet containing 45% protein for 4 weeks. At this stage, they were group weighed and feed various experimental diets containing 0.5% chromic oxide as an inert digestion indicator. After a ten day adjustment period, feces were collected from individual fish three times at five day intervals. Before the fecal collection fish were lightly anesthetized by immersion in seawater containing MS-222 (Ethyl m-aminobenzoate methanesultonate). Feces were collected from the rectal region by applying gentle pressure to the surface of the fish. Feces were freeze dried, ground and analyzed for nitrogen and chromic oxide.

The method of nitrogen determination has been already described. Chromic oxide in feed and feces was analyzed according to the method of Authur (1970). The apparent protein digestibility was calculated with the following formula:

$$\text{Apparent protein digestibility (\%)} = 100 - \left[100 \frac{\%Cr_2O_3 \text{ in feed}}{\%Cr_2O_3 \text{ in feces}} \times \frac{\% \text{ Protein in feces}}{\% \text{ Protein in feed}} \right]$$

The apparent digestibility of various proteins by Atlantic salmon is shown in Table 16. Casein was digested significantly better than FPC, soybean and fish meal protein. An increase in the level of protein showed a decrease in protein digestibility. However, the magnitude of decrease was much smaller for FPC and casein than fish meal and soy protein.

The difference in the digestibility of protein was not due to an amino acid imbalance because the deficient amino acids were added in the diet to overcome their deficiency according to the recommendation of NRC (1973). It is well accepted that the rate of food intake, the passage through digestive tract, the level of protein in the diet, the processing condition applied to the protein and the type of carbohydrate in the diet etc. influence the digestibility of protein in other animal species. These factors may have been responsible for the differences in the digestibility of various proteins. Furthermore, the presence of a high amount of salt in the fish meal may have been responsible for the lower digestibility of fish protein in seawater environment.

E. SUMMARY AND CONCLUSIONS

Three experiments were conducted to determine protein utilization by Atlantic salmon in seawater. In the first experiment, the dietary protein requirement was determined at 15 C by feeding five diets containing protein at equally spaced concentrations between 35 and 55% of the diet. The test protein was a mixture of casein and gelatin (80:20). The response criteria were growth, feed efficiency, mortality, body composition and liver composition. Based upon above criteria, the requirement was

estimated to be approximately 45 g protein per 100g of dry diet.

The second experiment was conducted to determine the effect of dietary calorie level on protein utilization by Atlantic salmon. Generally, the increase in dietary fat and protein level improved the growth and feed efficiency. Diet containing low protein, high carbohydrate and high fat resulted in increased liver glycogen deposition. A dietary balance of carbohydrate, lipid and protein was essential to overcome the high liver glycogen accumulation. Growth and biochemical data suggest that diet containing 45% protein and 20% fat showed the optimum response. The results also indicate that lipid was more efficiently utilized for energy purposes than carbohydrate. Further studies on energy utilization from commonly available carbohydrate and lipid sources of feed will be extremely useful in developing optimum calorie and protein ratios in practical feed formulations for salmon.

The third experiment was designed to investigate the digestibility of several dietary proteins by Atlantic salmon employing an indirect method using chromium oxide as digestion indicator. Casein was digested significantly better than fish protein concentrate (FPC), soybean and fish meal. An increase in the level of protein showed a decrease in protein digestibility. Fish meal and soybean protein showed much higher decreases in protein digestibility than FPC and casein with their increase in the level of diet. The difference in the digestibility of the proteins was not due to an amino acid imbalance because the diets were supplemented with the deficient amino acids.

The present study has demonstrated the minimum protein requirements of Atlantic salmon and the effect of carbohydrate and lipid on protein utilization. This information will be useful in the development of appropriate calorie and protein ratios for more efficient conversion of feed in the flesh of salmonids. Additional studies on amino acid requirements, amino acid availability and the factors influencing the digestibility of protein (eg. salinity, mineral content of food, type and amount of carbohydrate and lipid etc.) will provide a better understanding of protein utilization by salmonids in marine environment.

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TABLE 1. Composition of the Basal Diet[1].

INGREDIENT	AMOUNT (%)
Casein [2]	41.70
Gelatin	13.30
Dextrin	21.15
Vitamin Mixture[3]	4.00
Mineral Mixture [4]	5.00
α- Cellulose [5]	3.85
Amino Acid Mixture[6]	1.00
Herring Oil	10.00

[1]All diets contained 50% protein.

[2]Vitamin-free casein

[3]Vitamins added to supply the following (mg or I.U./kg of diet): Thiamine . Hcl, 50; riboflavin, 200; pyridoxine . Hcl, 50; d-calcium pantothenate, 300; d-biotin, 5; folic acid, 15; niacin, 500; cyanocobalamin, 0.2; L-ascorbic acid, 1000; inositol, 2000; choline chloride, 5000; para amino benzoic acid, 400; vitamin A, 5000 I.U.; vitamin D_3, 1500 I.U.; DL-alphatocopherol acetate, 300 I.U.; vitamin K, 40; butylated hydroxy toluene, 22.

[4]Composition of mineral mixture used in each diet is shown in Table 2.

[5]Diet 1, contained 8.85% alphacellulose because no mineral was added in this diet.

[6]Amino acid mixture supplied 0.5% Arginine.Hcl and 0.35% DL-methionine.

TABLE 2. Composition of the Mineral Supplements[1].

MINERAL	DIET #2	DIET #3	DIET #4	DIET #5	DIET #6
Calcium (%)	1.00	-	-	1.00	1.00
Phosphorous (%)	0.60	-	0.60	0.60	0.60
Magnesium (ppm)	-	-	-	-	600
Manganese (ppm)	-	100	100	100	100
Zinc (ppm)	-	18	18	18	18
Cobalt (ppm)	-	3.6	3.6	3.6	3.6
Copper (ppm)	-	72	72	72	72
Iron (ppm)	-	50	50	50	50
Sodium (ppm)	-	44	2270	44	864
Potassium (ppm)	-	0.1	3790	0.1	1953
Iodine (ppb)[2]	-	30	30	30	30
Fluorine (ppb)	-	29	29	29	29

[1]Mineral levels in Diet #6 is based on Bernhart-Tomarelli salt mixture except for the modifications in the calcium level.

[2]ppm or ppb per kg of diet.

TABLE 3. Average body weight, weight gain, feed conversion and mortality of fish fed various experimental diets.[1]

Diet no.	Average initial weight (g)	Average final weight (g)	Average weight gain (g)	Feed/gain ratio	Mortality (%)
1	56.8[a]	260.2[a,b]	203.4[b]	1.02[a]	3.3
2	57.7[a]	256.3[b]	198.6[b]	0.94[b]	4.0
3	56.9[a]	237.0[c]	180.0[c]	0.92[b]	4.0
4	57.1[a]	275.4[a]	218.3[a]	0.86[c]	0
5	57.3[a]	260.3[a,b]	203.0[b]	0.91[b,c]	5.0
6	57.4[a]	254.6[b]	197.2[b]	0.93[b]	3.0

[1]Treatment followed by the same letter are not significantly different ($P < 0.05$)

TABLE 4. The body composition of fish fed various experimental diets.

	Percentage				
Diet No.	Moisture	Protein	Lipid	Ash	Bone Ash
1	71.8 ± 0.17	17.2 ± 0.14	8.9 ± 0.67	1.68 ± 0.06	34.6 ± 0.64
2	71.4 ± 0.22	18.0 ± 0.07	7.9 ± 1.29	2.20 ± 0.03	45.4 ± 0.83
3	71.9 ± 0.11	17.4 ± 0.09	8.4 ± 0.38	1.97 ± 0.02	38.2 ± 0.57
4	71.9 ± 0.24	17.6 ± 0.12	7.9 ± 0.61	2.36 ± 0.01	49.3 ± 0.55
5	72.0 ± 0.28	17.8 ± 0.24	7.5 ± 0.73	2.31 ± 0.02	48.1 ± 0.40
6	71.9 ± 0.11	17.6 ± 0.10	7.9 ± 0.35	2.33 ± 0.03	49.1 ± 0.51

[1]Mean ± standard error

TABLE 5. Serum calcium, phosphorous, magnesium, sodium and potassium levels of fish fed various experimental diets.

Diet No.	Milligrams per 100 millitres of serum				
	Calcium	Phosphorous	Magnesium	Sodium	Potassium
1	14.6 ± 0.22[1]	10.9 ± 0.12	0.35 ± 0.02	378.8 ± 6.88	10.4 ± 0.62
2	15.8 ± 0.12	16.2 ± 0.48	0.54 ± 0.03	400.0 ± 2.89	11.0 ± 0.37
3	14.8 ± 0.31	14.4 ± 0.31	0.44 ± 0.01	392.5 ± 2.50	10.0 ± 0.22
4	15.6 ± 0.08	17.1 ± 0.12	0.43 ± 0.02	387.5 ± 2.50	9.6 ± 0.18
5	15.3 ± 0.34	16.1 ± 0.24	0.39 ± 0.01	373.8 ± 1.25	9.4 ± 0.09
6	15.3 ± 0.32	16.4 ± 0.48	0.39 ± 0.00	376.2 ± 3.14	9.6 ± 0.21

[1] Mean ± standard error.

TABLE 6. Serum zinc, iron, cobalt, copper and manganese levels of fish fed various experimental diets.

Diet No.	Zinc (µg/100 ml.)	Iron (µg /100 ml.)	Cobalt (µg /100 ml.)	Copper (µg /100 ml.)	Manganese (µg/100 ml.)
1	1.10 ± 0.07[1]	44.5 ± 3.51	30.0 ± 0.74	95.8 ± 2.36	5.0 ± 0.22
2	1.18 ± 0.06	35.8 ± 2.22	30.2 ± 1.07	94.0 ± 1.73	5.2 ± 0.20
3	1.60 ± 0.10	58.5 ± 3.87	164.2 ± 12.63	107.2 ± 2.21	11.9 ± 0.52
4	1.57 ± 0.03	63.5 ± 5.32	165.5 ± 9.46	104.2 ± 2.75	8.1 ± 0.28
5	1.52 ± 0.06	63.2 ± 8.18	174.0 ± 14.67	105.5 ± 1.50	9.3 ± 0.40
6	1.70 ± 0.05	68.5 ± 3.42	179.5 ± 16.56	110.0 ± 1.29	10.1 ± 0.28

[1] Mean ± standard error.

TABLE 7. Hematocrit, hemoglobin and serum protein level of Atlantic Salmon fed various experimental diets.

Diet No.	Serum Protein (g./100 ml.)	Hematocrit (%)	Hemoglobin (g./100 ml.)
1	4.3 ± 0.18[1]	39.5 ± 0.45	7.29 ± 0.09
2	4.3 ± 0.08	40.6 ± 0.49	7.06 ± 0.04
3	4.2 ± 0.22	47.0 ± 0.13	8.89 ± 0.09
4	4.3 ± 0.44	48.2 ± 0.54	9.14 ± 0.12
5	4.1 ± 0.27	48.0 ± 0.58	9.17 ± 0.17
6	4.4 ± 0.31	48.8 ± 0.87	9.27 ± 0.16

[1]Mean ± standard error

TABLE 8. Composition of experimental diets used in experiment 1.

Ingredient	Diet number (%)				
	1	2	3	4	5
Casein [1,2]	30.44	34.78	39.13	43.48	47.83
Gelatin [2]	7.37	8.42	9.47	10.53	11.58
Dextrin	38.19	32.80	27.40	21.99	16.59
Vitamin Mixture [3]	2.00	2.00	2.00	2.00	2.00
Mineral Mixture [4]	3.00	3.00	3.00	3.00	3.00
α - cellulose	5.50	5.50	5.50	5.50	5.50
Carboxymethyl Cellulose	2.00	2.00	2.00	2.00	2.00
Amino Acid Mixture [5]	1.50	1.50	1.50	1.50	1.50
Herring Oil	10.00	10.00	10.00	10.00	10.00

[1]Vitamin free casein. A product of ICN Pharmaceuticals, Inc., Cleveland, Ohio, U.S.A.

[2]Casein and gelatin supplied 80% and 20% protein respectively

[3]Vitamins added to supply the following (mg or I.U./kg of diet): thiamine.HCl, 50; riboflavin, 200; pyridoxine, HCl, 50; d-calcium pantothenate, 300; d-biotin, 5; folic acid, 15; niacin, 500; cyanocobalamin, 0.2; L-ascorbic acid, 1,000; inositol, 2,000; choline chloride, 5,000; para amino benzoic acid, 400; vitamin A, 5,000 I.U.; vitamin D_3, 1,500; I.U., DL-alphatocopherol acetate, 300 I.U.,; vitamin K, 40; butylated hydroxy toluene, 22.

[4]Modified Bernhart-Tomarelli salt mixture. Composition is described in Nutrient requirements of domestic animals No. 11, Nutritional Requirements of Trout, Salmon and Catfish, National Academy of Sciences, Washington, D. C., 1971, p. 49.

[5]Amino acid mixture supplied 0.5% Arginine.HCl, 0.25% L-Phenylalanine, 0.5% L-cystine and 0.2% DL-methionine.

TABLE 9. Average weight gain, feed conversion and mortality of fish fed various experimental diets for 12 weeks.[1]

Diet No.	Dietary Protein Level	Average Initial Weight (g)	Average Weight Gain (g)	Feed/Gain Ratio	Mortality (%)
1	35	73.2[a]	166.8[b]	1.16[a]	1.6
2	40	72.2[a]	163.0[b]	1.10[a,b]	5.0
3	45	72.0[a]	170.4[b]	1.06[a,b]	1.6
4	50	73.8[a]	171.8[a,b]	1.01[b,c]	6.7
5	55	73.0[a]	180.7[a]	0.90[c]	3.3

[1]Treatments followed by the same letter are not significantly different (P.<0.05)

TABLE 10. Changes in the body composition of Atlantic salmon fed different levels of protein.

Diet No.	Dietary Protein Level	Percentage			
		Moisture	Protein	Lipid	Ash
1	35	73.0 ± 0.49[1]	16.4 ± 0.28	7.75 ± 0.26	2.17 ± 0.02
2	40	73.2 ± 0.41	16.5 ± 0.33	8.34 ± 0.10	2.26 ± 0.04
3	45	72.6 ± 0.14	17.0 ± 0.04	8.15 ± 0.14	2.28 ± 0.04
4	50	73.0 ± 0.12	17.4 ± 0.26	7.59 ± 0.24	2.25 ± 0.04
5	55	73.4 ± 0.28	17.6 ± 0.07	6.84 ± 0.31	2.31 ± 0.01

[1]Mean ± standard error.

TABLE 11. Changes in the liver composition of Atlantic salmon fed different levels of protein.

Diet No.	Dietary Protein Level	Percentage Wet Weight		
		Glycogen	Protein	Lipid
1	35	5.70 ± 0.67[1]	12.0 ± 0.35	3.49 ± 0.52
2	40	4.36 ± 0.66	12.3 ± 0.18	4.49 ± 0.34
3	45	3.88 ± 0.79	12.4 ± 0.37	3.96 ± 0.18
4	50	3.91 ± 0.33	12.7 ± 0.53	3.89 ± 0.62
5	55	3.92 ± 0.23	12.9 ± 0.47	3.94 ± 0.41

[1] Mean ± standard error

TABLE 12. Composition of experimental diets used in experiment 2.

Ingredient	Diet Number (%)								
	1	2	3	4	5	6	7	8	9
Casein [1,2]	30.44	30.44	30.44	39.13	39.13	39.13	47.83	47.83	47.83
Gelatin[2]	7.37	7.37	7.37	9.47	9.47	9.47	11.58	11.58	11.58
Dextrin	27.50	27.50	27.50	17.25	17.25	17.25	7.00	7.00	7.00
Vitamin Mixture[3]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral Mixture[4]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
α - cellulose	18.19	12.19	6.19	17.65	11.65	5.65	17.09	11.09	5.09
Carboxymethyl Cellulose	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Amino Acid Mixture[5]	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Herring Oil	8.00	14.00	20.00	8.00	14.00	20.00	8.00	14.00	20.00

1, 2, 3, 4 and 5 - See footnote Table 8.

TABLE 13. Average weight gain, feed conversion and mortality of Atlantic salmon fed various experimental diets for 12 weeks.

Diet						
Diet No.	Protein Level (%)	Lipid Level (%)	Average Initial Weight (g)	Average Weight Gain (g)	Feed/Gain Ratio	Mortality (%)
1	35	8	58.8	134.4	1.16	8.3
2	35	14	58.6	136.4	1.10	5.0
3	35	20	57.1	144.2	1.07	8.3
4	45	8	58.8	136.4	1.09	8.3
5	45	14	57.1	140.8	0.99	5.0
6	45	20	57.4	150.4	0.90	8.3
7	55	8	57.3	150.1	0.92	6.6
8	55	14	57.4	150.6	0.86	5.0
9	55	20	57.0	161.2	0.82	0.0

TABLE 14. Changes in the body composition of fish fed various experimental diets.

Diet			Percentage			
Diet No.	Protein Level	Lipid Level	Moisture	Protein	Lipid	Ash
1	35	8	74.6 ± 0.14[1]	17.3 ± 0.23	5.8 ± 0.23	2.28 ± 0.03
2	35	14	72.7 ± 0.37	16.4 ± 0.07	8.6 ± 0.46	2.30 ± 0.03
3	35	20	71.6 ± 0.44	16.0 ± 0.15	9.9 ± 0.25	2.31 ± 0.03
4	45	8	75.5 ± 0.08	16.8 ± 0.11	5.4 ± 0.04	2.34 ± 0.06
5	45	14	73.8 ± 0.18	16.9 ± 0.32	6.9 ± 0.21	2.34 ± 0.10
6	45	20	71.5 ± 0.32	16.7 ± 0.07	9.4 ± 0.51	2.26 ± 0.06
7	55	8	74.5 ± 0.22	17.2 ± 0.12	6.0 ± 0.19	2.30 ± 0.04
8	55	14	73.4 ± 0.10	17.1 ± 0.37	7.3 ± 0.06	2.22 ± 0.06
9	55	20	71.5 ± 0.37	17.2 ± 0.08	8.6 ± 0.22	2.18 ± 0.05

[1]Mean ± standard error

TABLE 15. Changes in the liver composition and serum protein and glucose levels of Atlantic salmon fed various experimental diets.

Diet			Liver (Percentage Wet Weight)			Serum	
No.	Protein Level	Lipid Level	Protein	Glycogen	Lipid	Protein (g/100 ml.)	Glucose (mg/100 ml.)
1	35	8	12.5 ± 0.47[1]	3.30 ± 0.42	3.45 ± 0.40	4.35 ± 0.18	237.7 ± 9.83
2	35	14	12.1 ± 0.31	4.31 ± 0.19	3.73 ± 0.13	4.52 ± 0.20	266.0 ± 3.67
3	35	20	12.2 ± 0.41	4.89 ± 0.44	3.76 ± 0.15	4.42 ± 0.14	261.5 ± 11.73
4	45	8	12.6 ± 0.29	3.12 ± 0.16	3.70 ± 0.23	3.98 ± 0.19	241.7 ± 8.45
5	45	14	12.7 ± 0.40	3.08 ± 0.16	3.78 ± 0.36	4.22 ± 0.16	248.2 ± 14.21
6	45	20	12.7 ± 0.40	3.50 ± 0.28	3.64 ± 0.12	4.32 ± 0.07	252.0 ± 16.76
7	55	8	13.0 ± 0.42	2.22 ± 0.17	3.40 ± 0.10	4.09 ± 0.11	244.3 ± 1.94
8	55	14	13.3 ± 0.53	2.27 ± 0.21	3.50 ± 0.22	4.37 ± 0.08	221.8 ± 7.32
9	55	20	13.1 ± 0.29	3.06 ± 0.17	3.79 ± 0.29	3.95 ± 0.21	223.3 ± 8.21

[1]Mean ± standard error

TABLE 16. Digestibility of various proteins by Atlantic salmon in seawater.

Test Protein	Diet No.	Dietary Protein (%)	Digestibility of Protein (%)
Casein	1	35	94.8 ± 0.37[1]
	2	45	93.4 ± 0.29
Soybean Protein	3	35	88.5 ± 0.47
	4	45	84.3 ± 0.92
Fish Protein Concentrate	5	35	91.8 ± 0.32
	6	45	91.2 ± 0.22
Fish Meal	7	35	79.1 ± 0.48
	8	45	76.3 ± 0.98

[1]Mean ± standard error

POOR

QUALITY

ORIGINAL